

CLAIMS

1. A recombinant plasmid expression vector comprising:
 - a) at least one gene sequence of a mesophilic bacterium coding for a polypeptide having enzyme UdP activity and/or enzyme PNP activity; and
 - b) at least one gene sequence coding for antibiotic resistance.
2. A plasmid vector according to claim 1, characterised in that the at least one sequence coding for antibiotic resistance is a sequence coding for tetracycline and/or kanamycin and/or ampicillin resistance.
3. A plasmid vector according to claim 1, characterised in that it comprises both the sequence coding for the polypeptide having enzyme UdP activity and that coding for the polypeptide having enzyme PNP activity.
4. A plasmid vector according to claims 1 to 3, characterised in that the at least one gene sequence encoding a polypeptide having enzyme UdP activity and/or enzyme PNP activity and the gene sequence coding for tetracycline and/or kanamycin resistance are cloned into the plasmid pUC18.
5. A plasmid vector according to claim 1, characterised in that the mesophilic bacterium is *E.coli*.
6. A plasmid vector according to claim 5, characterised in that the sequence encoding a polypeptide having enzyme UdP activity is the sequence *udp*.
7. A plasmid vector according to claim 6, characterised in that the sequence is the EMBL sequence X15689.
8. A plasmid vector according to claim 5, characterised in that the sequence encoding a polypeptide having enzyme PNP activity is the sequence *deoD*.
9. A plasmid vector according to claim 8, characterised in that the sequence is the EMBL sequence M60917.
10. A plasmid vector according to claims 1 and 4, characterised in that the sequence coding for tetracycline resistance is the Tet gene of pBR322.

11. A plasmid vector according to claims 1 and 4, characterised in that the sequence coding for kanamycin resistance is the kan gene of pET29c.
12. A plasmid vector according to claims 1 to 11, characterised in that said gene sequence coding for a polypeptide having enzyme UdP activity and said gene sequence coding for a polypeptide having enzyme PNP activity are fused together so to express a fusion protein wherein the enzymes UdP and PNP are covalently bonded together.
13. A plasmid vector according to claims 1 to 11, characterised in that said gene sequence coding for a polypeptide having enzyme UdP activity and said gene sequence coding for a polypeptide having enzyme PNP activity are fused together so to express a fusion protein having the formula UdP-(L)-PNP wherein L is a polypeptide linker of more than one aminoacidic units.
14. A plasmid vector selected from: pGM679 (SEQ ID NO 1), pGM708 (SEQ ID NO 2), pGM678 (SEQ ID NO 3), pGM707 (SEQ ID NO 4), pGM712 (SEQ ID NO 5), pGM716 (SEQ ID NO 6), pGM709 (SEQ ID NO 7), pGM769 (SEQ ID NO 8), pGM771 (SEQ ID NO 9), pGM795 (SEQ ID NO 10), pGM746 (SEQ ID NO 11), PGM747 (SEQ ID NO 12), pGM751 (SEQ ID NO 13), pGM800 (SEQ ID NO 14) and pGM807 (SEQ ID NO 15).
15. Prokaryotic host cells, characterised in that they contain at least one plasmid vector according to claims 1 to 14.
16. Host cells according to claim 15, characterised in that they are bacterial cells.
17. Host cells according to claim 16, characterised in that they are cells of *Escherichia coli*.
18. Host cells according to claim 17, characterised in that they are cells of strain K12, preferably MG1655 or DH5 α , and/or of strain B.
19. Use of host cells according to claims 15 to 18, or of the corresponding crude or purified extracts, either separately or in combination, in the production of polypeptides having enzyme UdP activity and/or enzyme PNP activity.

20. Use of host cells according to claims 15 to 18, or of the corresponding crude or purified extracts, either separately or in combination, as catalysts of transglycosylation reactions between a donor nucleoside and an acceptor base.
21. Use according to claim 20, characterised in that the acceptor base is a purine and/or pyrimidine base.
22. Use according to claim 21, characterised in that the purine and/or pyrimidine bases are selected from natural or substituted pyrimidine and purine bases; purine bases substituted in the 1, 2 and/or 6 positions; pyrimidine bases substituted in the 3 and/or 5 positions; purine, 2-azapurine, 8-azapurine and substituted analogues thereof, 1-deazapurine (imidazopyridine), 3-deazapurine, 7-deazapurine and substituted analogues thereof.
23. Use according to claim 21, characterised in that the acceptor bases are constituted by heterocyclic compounds containing at least one nitrogen atom, such as, for example, imidazoles and substituted analogues thereof, triazoles and substituted analogues thereof and pyrazoles and substituted analogues thereof.
24. Use according to claim 21, characterised in that the donor nucleoside is selected from natural and/or modified nucleosides containing D-ribose and 2'-deoxyribose; nucleosides containing the ribose group modified in the 2', 3' and/or 5' positions; nucleosides in which the sugar is β -D-arabinose, α -L-xylose, 3'-deoxyribose, 3',5'-dideoxyribose, 2',3'-dideoxyribose, 5'-deoxyribose, 2',5'-dideoxyribose, 2'-amino-2'-deoxyribose, 3'-amino-3'-deoxyribose, 2'-fluoro-2'-deoxyribose.
25. Use of host cells according to claims 15 to 18, either separately or in combination, or of the corresponding crude or purified extracts, in the preparation of nucleoside analogues containing heterocyclic systems having purine and/or pyrimidine bases substituted by one or more nitrogen atoms.
26. Use of host cells according to claims 15 to 18, either separately or in combination, or of the corresponding crude or purified extracts, in the preparation of α -pentose-1-phosphate sugars by phosphorolysis reactions.

27. Use of host cells according to claims 15 to 18, either separately or in combination, or of the corresponding crude or purified extracts, in the production of nucleosides and modified analogues thereof.

28 A method for producing a fusion protein having the activity of both UDP and PNP enzymes, said method comprising:

- (a) producing a plasmid expression vector according to claims 12 or 13;
- (b) transforming a host bacteria cell with said expression vector; and
- (c) isolating and purifying the fusion protein from the transformed bacteria cell.

29. A method according to claim 28 characterised in that said host bacteria cells are cells of *Escherichia coli*.

30. A fusion protein having the activity of both UdP and PNP enzymes obtainable from the method according to claims 28-29.

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